Supplemental Figure 1

A. The procedure followed for making the detergent spin columns used in this study. The stationary phase is removed from a commercial SDS removal spin column (Pierce Protein Research Products, Part # 87777) and approximately 100 µL (~25 mg of stationary phase) of slurry is loaded back into the spin column. Material was loaded onto the self-packed spin column and incubated at RT for 2 minutes. The digest was recovered by centrifugation at 1500g for 1.5 minutes. This procedure was effective at removing 0.1% SDS from 50 µL of sample. It is worth noting at higher protein loads (>50 µg) we observed a small amount of SDS eluting during the wash phase (i.e., high organic). If needed, the amount resin can be increased to rectify the small amount of SDS observed at higher protein loads. However, the observed SDS did not negatively impact the chromatography or suppress ESI signal and furthermore recent reports have shown that LC-MS is tolerant of small amounts of SDS(REF 10). At lower protein loads (<25ug) no SDS was detected at high organic. **B.** A protein standard mix was spiked with 0.1% SDS and then subjected to SDS removal using different amounts of stationary phase. The plot shows the spectral counts of 5 different protein standards decrease as a function of increasing amounts of stationary phase. Minimal sample loss was associated with 25 mg of stationary phase. The sample marked as "no cleanup" was the same digest but with no SDS and therefore did not undergo any cleanup. **C**. Finally, twice as many peptides are identified from a complex mixture using the modified approach described here when compared to the commercial SDS spin column.

After submission of this manuscript, Pierce now sells the packing resin for removal of small amounts of SDS from protein mixtures (Product #87780).

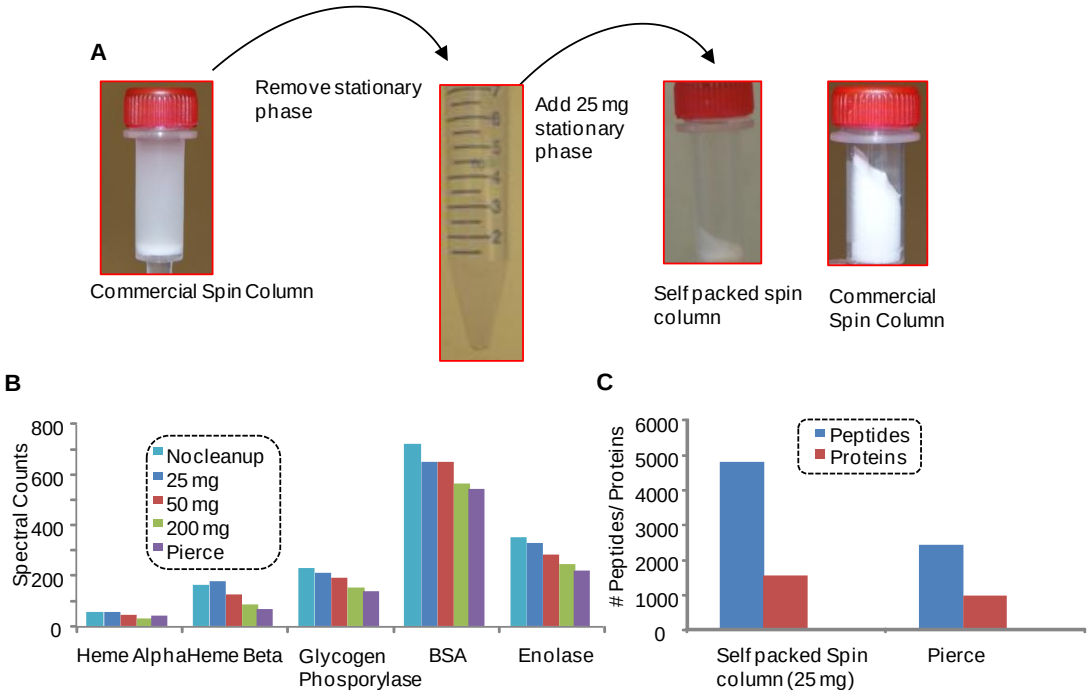
Supplemental Figure 2

The number of peptide spectral matches ($q<0.01$) between the FASP and SDS spin procedure for *S. cerevisiae*; **B)** *C. elegans*; and **C)** human. The SDS spin procedure returned statistically more peptide spectral matches for each organism than the FASP procedure using 1-tailed t test ($p<0.05$). Error bars correspond to 95% CI in the mean.

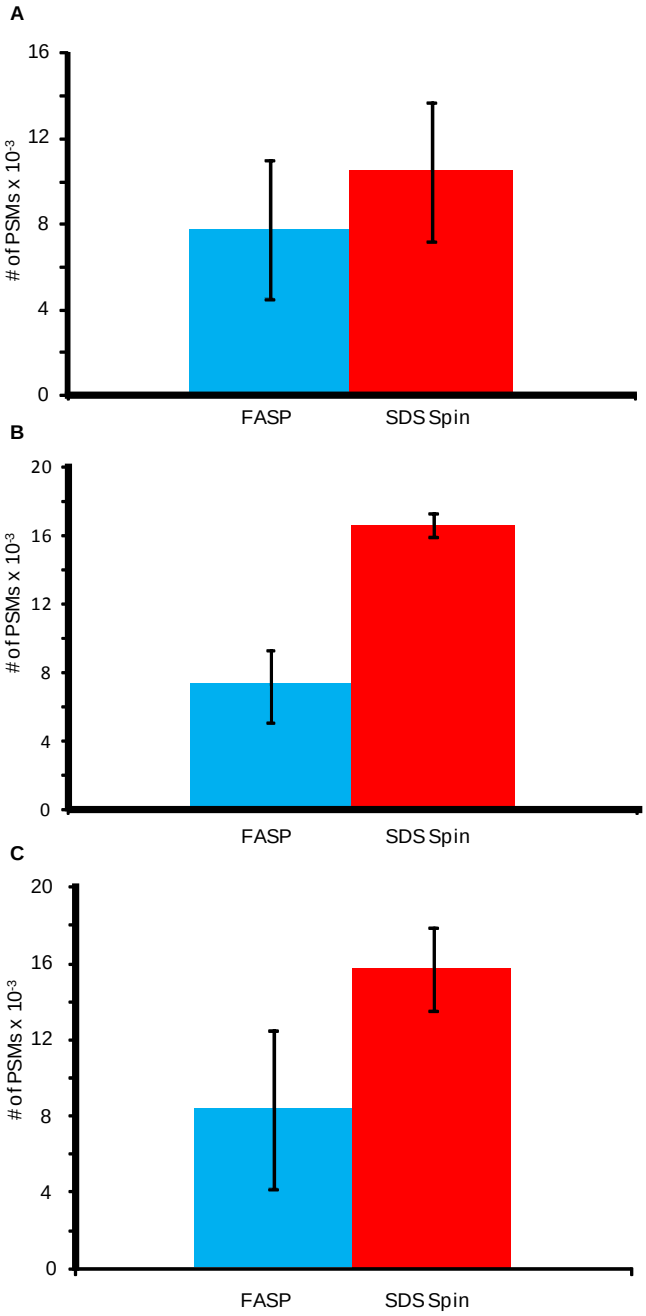
Supplemental Figure 3

Gene ontology annotations for cellular components of the proteins identified from **A)** *S. cerevisiae*; **B)** *C. elegans*; and **C)** human. The first column displays the number of proteins annotated for the different cellular compartments as a percentage of total proteins identified. The 2nd column compares the total number of proteins between the compartments for the different procedures. For example ~35% of proteins identified in the *C. elegans* sample were annotated to be membrane-associated proteins for both FASP and SDS spin column procedures; however, the SDS spin procedure identified on average approximately 30% more membrane proteins (**B**).

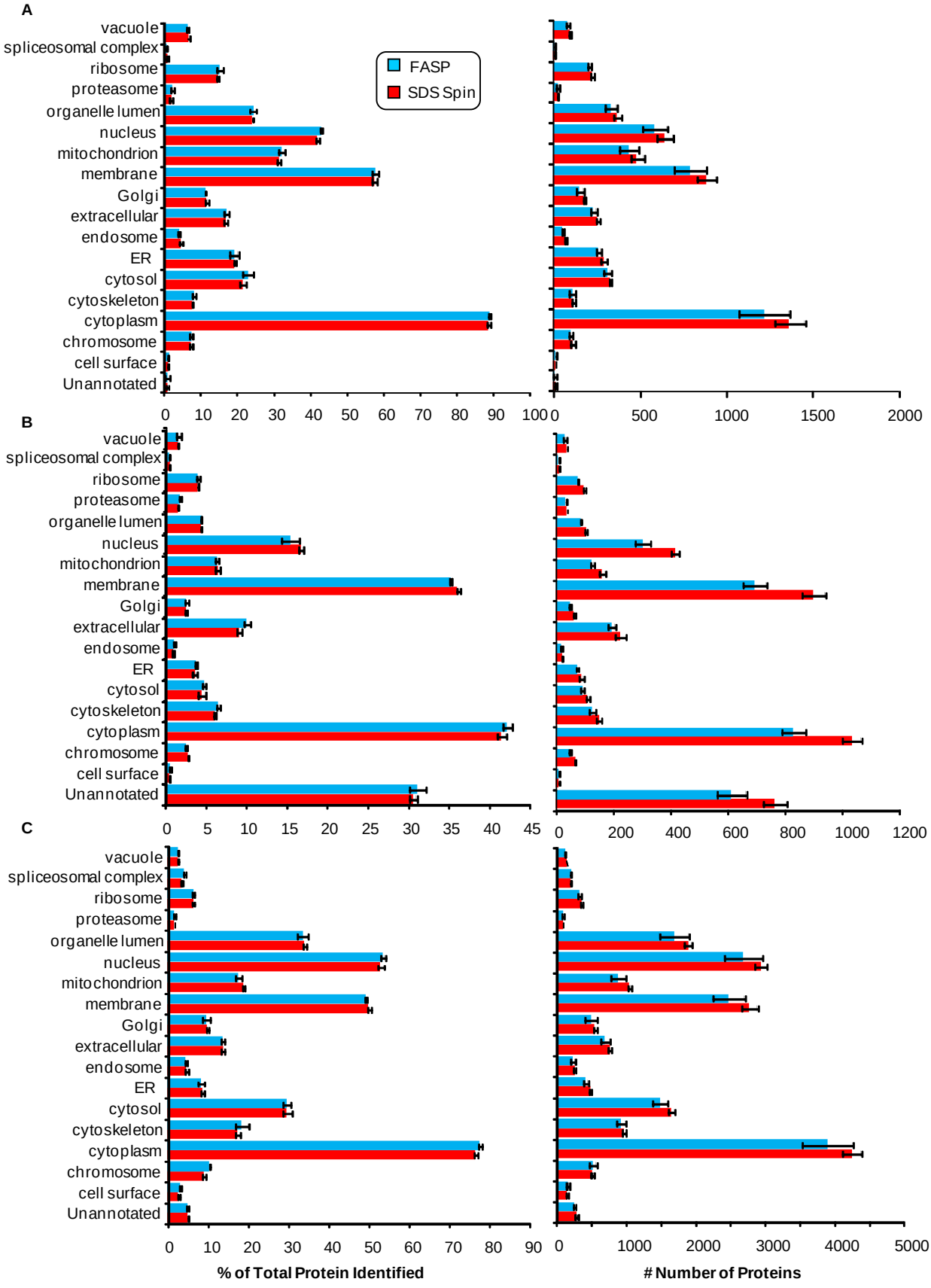
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Sample Preparation (Supplemental)

HEK293T

The SV40-transformed human embryonic kidney cell line HEK293T was obtained from the Coriell Institute Cell Repositories (Camden, NJ). Cells were grown in Dulbecco-modified Eagle's medium (MediaTech CellGro, Manassas, VA) containing 4,500 mg/L glucose and supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and penicillin and streptomycin sulfate (100 U/ml and 100 mg/ml, respectively; Invitrogen, Carlsbad, CA) in a humidified 37°C, 7% incubator. The cells were allowed to reach 90% density before collection. The pellet was washed in 1x PBS and frozen at -80°C prior to processing. This pellet was then subjected to digestion as described in the manuscript.

S. cerevisiae

Yeast (*Saccharomyces cerevisiae*, strain BY4741) were grown to an OD600 of ~0.7, and the cells were centrifuged at 3000*g*, 4 °C, for 5 min. The supernatant was resuspended in 1 mL of cold 50 mM ammonium bicarbonate buffer (pH 7.8), mixed with 1 mL of 0.5 mm glass beads, and lysed with a mini-beadbeater (Biospec Products Inc., Bartlesville, OK) for three 1 min repeats. The suspension was kept on ice for 1 min, then centrifuged at 2000*g* at 4 °C for 10 min to separate the supernatant from beads and debris and then centrifuged at 14000*g* at 4 °C for 10 min to separate insoluble material. This fraction was then subjected to digestion as described in the manuscript

C. elegans

C. elegans (N2 strain) were grown on enriched peptone plates seeded with the OP50 strain of *E. coli* at 20 °C. Worms of all developmental stages were washed from the plates with M9 buffer (22 mM KH_2PO_4, 22 mM Na_2HPO_4, 85 mM NaCl, 1 mM $MgSO_4$; VWR, West Chester, PA) and sucrose floated to remove bacterial contamination. The worms were then lysed in 50 mM ammonium bicarbonate pH 7.8 using the small probe of a sonic dismembrator model 100 (Thermo Fisher Scientific, Pittsburgh, PA) for 5 cycles of a 20 s continuous pulse followed by a 60 s ice incubation. The lysate was then centrifuged at 4000 rpm for 10 min at 4 °C in an Eppendorf 5417R microcentrifuge (Westbury, NY) to remove cell debris. A second centrifugation at 14000 rpm for 10 min at 4 °C is then performed to separate the soluble lysate from the insoluble lysate. The soluble lysate was subjected to digestion as described in the manuscript.

Analysis Pipeline

Sequest version 2.7 was used to search the data with the following parameters: one static modification (Cys 57.0 Da); No dynamic modifications; and a Precursor mass tolerance ±3.0 Da. The data were searched using the average mass for precursor data and monoisotopic data for ms^2 spectra using the IPI human database (v 3.57, 76541 ORFs, 47 contaminants), *C. elegans* (wormbase, WS160, 23141 ORFs, 47 contaminants, *S. cerevisiae* (SGD ORF database 2/20/2009, 6717 ORFs, 47 contaminants for each corresponding organism. The target and decoy database search results were processed by Percolator[5] to improve peptide-spectrum matches and enforce a peptide level *q* value threshold of ≤0.01.